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Effect of Application Rate on the Sensitivity of *Erysiphe graminis* f.sp. *tritici* to Fenpropimorph*

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Abstract: Two sprays of fenpropimorph per season were applied to a winter wheat field trial, at a range of rates, which included the full commercial rate, in order to test the effect of fenpropimorph sprays on the sensitivity of *Erysiphe graminis* f.sp. *tritici*. While a reduction in the sensitivity of the mildew isolates was detected after fungicide application, this was not dependent on the rate of fungicide applied. Reduced rates were not found to induce a larger shift towards insensitivity than the full commercial rate. Powdery mildew isolates were collected from 1993 to 1996 and their sensitivity to fenpropimorph determined in order to monitor sensitivity changes in the population. While a decline in sensitivity was noted from season to season, there was no correlation between a lower sensitivity and the rate of fenpropimorph previously used. Isolates collected in Scotland were found to be significantly less sensitive than those sampled in the south of England.

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1 INTRODUCTION

After the development of resistance to some of the early systemic fungicides used for the control of wheat and barley powdery mildew caused by *Erysiphe graminis* DC, such as the demethylation inhibitors and aminopyrimidines,[1–4] control of powdery mildew in cereals has relied almost exclusively on one class of fungicide, the most commercially important members of which are the two morpholines, fenpropimorph and tridemorph, and one piperidine, fenpropidin. The group is commonly referred to as the morpholines and they act by inhibiting ergosterol synthesis from lanosterol to differing degrees at three or more sites.

With reliance for wheat mildew control resting on a single group of fungicides, the risk of insensitivity in the target population causes concern despite the multi-site action of the morpholines, and monitoring programmes have been carried out since the introduction of these chemicals.[7] Sensitivity in the mildew population to the morpholines has remained high,[8] but in recent seasons several surveys have reported a decline in the sensitivity of isolates in Europe to fenpropimorph,[9,10] although at the present time field control remains good.

Morpholines are usually applied in mixtures, which is a recommended part of an anti-resistance strategy.[11] Another standard practice, however, is to reduce the rate applied. Reduced rates can give good mildew control and reduce the input costs to the crop,[12] but there is the possibility that such reduced rates could influence the likelihood of resistant isolates arising in the mildew population. Larger reductions in sensitivity

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following multiple split applications rather than following fewer full-rate applications have been reported,[13–15] but the influence of reduced rates *per se* is not clear.

This paper reports the results of a field experiment carried out in 1995 as well as data gathered from a survey of mildew isolates collected in the United Kingdom over a period of several years. Isolates from both investigations were tested for sensitivity to fenpropimorph to establish whether any shift in sensitivity could be measured following a repeat application of reduced rates of fenpropimorph, as applied in standard practice.

2 MATERIALS AND METHODS

A winter wheat (*Triticum aestivum* L.) trial was planted in the autumn of 1994 at Boghall Farm on the Bush Estate in the Midlothian region of Scotland. The cultivar used was Mercia. Plot sizes were 24 × 16 m. Fertiliser, herbicide and any micro-nutrient treatments applied were uniform across all plots, and accorded with local practice. The seed was treated with guazatine.

Fungicide treatment consisted of two spray applications of fenpropimorph at one of three reduced rates or the full commercial rate (Table 1). The first fungicide application was made when mildew (*E. graminis* f.sp. *tritici* Marchal) first developed on the plants and the second spray was applied three weeks later. To reduce uncontrolled variation in mildew isolates as a result of freely mobile inoculum from untreated plots, there were no unsprayed plots. The field used for the trial was surrounded on three sides by uncultivated upland and woods, and the site was therefore isolated from neighbouring wheat fields. All fungicides were applied in 270 litre ha^{-1} water using a tractor-mounted Allman hydraulic sprayer with standard flat fan nozzles at a pressure of two bars.

Infected leaves were sampled from the middle of plots at three times during the season; just prior to spraying and three weeks after both the first and second sprays. Leaves were sampled from as low down in the crop canopy as possible on the premise that these leaves would have received the lowest doses of fungicide. The levels of mildew in the plots were recorded at the time of sampling, by assessing the percentage surface area on each of the top three leaves infected with mildew on a sample of ten plants taken from the central area of the plots.

TABLE 1
Fungicide Programmes Evaluated

Treatment Reference[a]	*First application 2 June 1995 GS 39-43*	*Second application 28 June 1995 GS 66*
1·0	Fenpropimorph 1·0	Fenpropimorph 1·0
0·75	Fenpropimorph 0·75	Fenpropimorph 0·75
0·5	Fenpropimorph 0·5	Fenpropimorph 0·5
0·25	Fenpropimorph 0·25	Fenpropimorph 0·25

[a] Fenpropimorph was applied as 'Corbel' 750 g litre^{-1} EC; the treatment number represents the proportion of the full commercial rate (750 g AI ha^{-1}) used.

Bulk isolates collected from leaves from each plot were tested for sensitivity to fenpropimorph in the laboratory using a method adapted from one used to assess the sensitivity of *E. graminis* f.sp. *hordei* Marchal.[16] Mildew pustules from 10–15 leaves were bulked on whole plants of the variety Cerco, which carries no known mildew resistance genes, and maintained in an isolation propagator to prevent cross-contamination. To determine the sensitivity of isolates in tests, seedlings of Cerco were grown to the second true leaf stage and fenpropimorph solutions were then applied at doses of 0·058, 0·117, 0·234, 0·469 and 0·938 g litre^{-1} in a spray cabinet using a Humbrol spray gun for 10 s. Control plants were sprayed with water. Each spray treatment was replicated using the same spray cabinet. Treated sets of plants were kept apart for 24 h before the preparation of leaf segments. Eight segments (2 cm long) were cut from the second true leaf of the treated plants from each concentration and spray cabinet combination and then plated on Davis minimal medium containing benzimidazole (80 mg litre^{-1}) and inoculated with the experimental isolates. Inoculation was carried out by dusting the mildew inoculum from the heavily infected plants evenly over the surface of the leaf segment using a fine, sterile paint brush. The percentage mildew cover after incubation for 14 days at 18°C and 24 h light per day was assessed visually and the data analysed using a Genstat 5 programme which fitted symmetrical logistic curves and allowed median efficacy (EC_{50}) values to be calculated.

Additional mildew isolates collected from crops throughout the east of Scotland and East Anglia, England, were assayed for sensitivity to fenpropimorph. Survey isolates were taken from within commercial crops or from field trials, and the fungicide history of the crop, variety, area and date of collection were recorded. These isolates were bulked from single pustules and tested for fenpropimorph sensitivity as described above. The sensitivity of these isolates was then correlated with their known history.

3 RESULTS

3.1 Field trial results

The results for the field experiment are summarised in Tables 2, 3 and 4. There was a significant ($P = 0{\cdot}049$) decrease in the sensitivity of isolates collected after the first sprays had been applied. Although the sampled iso-

TABLE 2
Sensitivity of Isolates from 1995 Field Experiment to Fenpropimorph

	EC_{50} *values* (*g litre*$^{-1}$) *Fenpropimorph treatments*			
Sampling time	*1·0*	*0·75*	*0·5*	*0·25*
Before spray	0·263	0·131	0·209	0·193
After one spray	0·348	0·263	0·274	0·328
After two sprays	0·253	0·136	0·173	0·197
SED				0·0934 $P = 0{\cdot}049$

lates varied in their sensitivity to fenpropimorph, there were no significant differences between mean EC_{50} values for isolates in relation to the concentration of fungicide to which they had been exposed (Table 2). The mean sensitivity to fenpropimorph for isolates collected after the second spray application was not significantly different from the mean values at the beginning, prior to any sprays being applied; however the range of sensitivities at this last timing was greater than at the other two sampling times, so that isolates with significantly reduced sensitivity remained within this sample group (Table 3). Assessment of the levels of mildew present in the plots at each assessment timing (Table 4) revealed that there was a large influx of mildew into the plots after the second spray, which may have introduced more sensitive 'wild type' isolates from the general mildew population into the trial plot population.

TABLE 3
Sensitivity of Isolates from 1995 Field Experiment to Fenpropimorph

	EC_{50} (*g litre*$^{-1}$)	
Sampling time	*Range*	*Mean*
Before spray	0·027–0·310	0·199
After one spray	0·161–0·441	0·303
After two sprays	0·021–0·448	0·190
SED		0·0467 $P = 0{\cdot}049$

3.2 Survey results

Analysis of the fenpropimorph sensitivity of mildew isolates collected between 1993 and 1996 as part of a survey for fungicide sensitivity in Scotland are shown in Table 5. The sensitivity of the isolates to fenpropimorph was compared to the known fungicide history of the isolates. There was no indication that previous treatment with fenpropimorph had significantly influenced the sensitivity of the isolates collected ($P = 0{\cdot}794$), or that the rate applied had influenced the sensitivity of the isolates collected ($P = 0{\cdot}624$).

The survey data gathered between 1993 and 1996 show a decline in the mean sensitivity to fenpropimorph from season to season (Table 6) with a significant decrease ($P < 0{\cdot}001$) in sensitivity between 1994 and 1995. This shift in the mean sensitivity of isolates was continued into the following year (1996) though no further significant decrease was detected.

Significant differences in sensitivity were detected in the survey isolates when the geographical source of the isolates was considered (Table 7). The analysis of EC_{50}

TABLE 5
Sensitivity of Survey Isolates to Fenpropimorph

Fenpropimorph treatment	EC_{50}(*g litre*$^{-1}$)($\pm SD$)
Full dose	0·249 (±0·133)
Half dose	0·226 (±0·101)
Quarter dose	0·221 (±0·135)
Nil	0·342 (±0·142)
Pooled SD	(±0·133)

TABLE 4
Mean Percentage Mildew Cover on Top Three Leaves

	Before spray GS 39–43 Leaf			*After one spray GS 66 Leaf*			*After two sprays GS 73 Leaf*		
Treatment	*1*	*2*	*3*	*1*	*2*	*3*	*1*	*2*	*3*
1·0	0	0	1·1	0	0	1·3	0·1	8·8	7·9
0·75	0	0	0·6	0	0·1	0·9	0·2	3·8	10·8
0·5	0	0	2·2	0	0	2·2	2·5	7·0	9·3
0·25	0	0	0·1	0	0	1·5	0·1	6·3	14·0
SED	0 n.s.	0 n.s.	0·95 n.s.	0 n.s.	0·56 n.s.	0·60 n.s.	0·92 n.s.	3·02 n.s.	4·29 n.s.

TABLE 6
Sensitivity to Fenpropimorph in Isolates of Powdery Mildew Collected from 1993 to 1996

		EC_{50} (*g litre*$^{-1}$)	
Year	*Mean*	*Range*	*SD*
1993	0·167	0·012–0·244	±0·081
1994	0·185	0·062–0·324	±0·083
1995	0·327	0·163–0·548	±0·124
1996	0·341	0·185–0·546	±0·128
Pooled SD			±0·107

data revealed that isolates collected in Scotland were significantly less sensitive to fenpropimorph than isolates collected in East Anglia ($P = 0{\cdot}006$).

There were no significant differences between the mean EC_{50} values of isolates derived from the following varieties:- Apollo, Mercia, Rialto, Riband, Lynx, Hunter, Hereward and Beaver ($P = 0{\cdot}263$). The month in the year in which the isolates was sampled did not significantly influence the sensitivity ($P = 0{\cdot}186$).

4 DISCUSSION

4.1 Reduced rates

A decline in the sensitivity of mildew isolates to fenpropimorph was detected in the field experiment after fenpropimorph had been applied; however this shift in sensitivity was not related to the rate applied and there was no evidence that reduced rates increased or decreased the sensitivity of the mildew isolates assayed. Even after two sprays of fenpropimorph, when more 'wild type' mildew inoculum invaded the plots, the less-sensitive isolates could still be detected in the field trial mildew population. A shift following the use of fenpropimorph could not be detected in the survey data, probably as a result of the high degree of variation in the population. These findings, that reduced rates of fenpropimorph did not pose an increased risk of insensitivity arising in *E. graminis* f.sp. *tritici*, were also found for barley mildew, *E. graminis* f.sp. *hordei*, where no correlation between rate of fenpropimorph and sensitivity has been found in field trials,[17] but exposure to fenpropimorph, regardless of rate, did cause an increase in the frequency of 'resistance' in the barley mildew isolates sampled.[18]

TABLE 7
Sensitivity of Isolates to Fenpropimorph Related to Sampling Area

Isolate source	EC_{50} (*g litre*$^{-1}$) (±*SD*)
Scotland	0·271 (±0·128)
East Anglia	0·165 (±0·087)
Pooled SD	(±0·119)

Although the findings presented in this paper indicate that reduced rates do not cause an increased risk of resistance, multiple split applications of fenpropimorph, alone or in a mixture with propiconazole, have been shown to cause larger reductions in sensitivity than fewer full-rate applications.[13,14] This has been shown even where the total amount of fenpropimorph applied was equal in both regimes.[15] The degree of mildew control has been shown to be critical in determining the size of the shift in sensitivity, probably as a result of the selection pressure exerted by improved control.[15] Several other authors of papers where shifts are reported in response to split applications also comment that disease control was superior in these treatments.[13,14] In the trial reported here the levels of mildew assessed in the plots show that disease control was as good in the reduced-rate plots as in the full-rate plots, so that this selection pressure, if it exists, was equal in all treatments.

Multiple split applications of fenpropimorph can cause a larger reduction in sensitivity than a lower number of full-rate sprays,[13–15] and the work would lead to the conclusion that it may have been the multiple and continuous nature of the applications, and, therefore, the selection pressure, rather than the dose, that was critical. Standard practice in the United Kingdom is to reduce the rate from the full commercial rate when controlling mildew, to reduce input costs, rather than to increase the number of applications made to the crop. In addition, the maximum number of applications for many formulated products containing fenpropimorph is restricted in the United Kingdom to two or three depending on the formulated product used,[19] so that multiple applications of reduced rates are seldom applied. Some formulated products containing either fenpropimorph, fenpropidin or tridemorph do permit a maximum total dose, rather than a maximum application number, to be applied, which would allow multiple applications to be made.

If the degree of mildew control determines the size of the shift in sensitivity to fenpropimorph and if control is sometimes inferior with reduced rates where the persistence of the fungicide can be shorter,[20,21] then, in theory, the selection pressure from reduced rates may actually be less than for highly effective full-rate sprays.

Survey data indicated that geographical location was much more influential on the sensitivity of isolates tested. English isolates assayed as part of the annual survey at SAC were significantly more sensitive that Scottish isolates. This could be due to differences in cultural practices between the two areas. The variety Riband is the predominant variety in Scotland and, as it

yields well in response to fungicides, inputs therefore tend to be high.

4.2 Cross-resistance to other fungicides

Cross-resistance exists in barley powdery mildew, *E. graminis* f.sp. *hordei*, between fenpropimorph and fenpropidin, whereas sensitivity to tridemorph is considered to be negatively associated with these two.[18,22] Preliminary studies with *E. graminis* f.sp. *tritici* on a limited number of isolates have indicated that fenpropimorph could be moderately cross-resistant to fenpropidin with a low level or no cross-resistance to tridemorph.[9,23] Cross-resistance would hasten the decline in sensitivity to the other morpholines within the group, but is not in keeping with survey data that show no corresponding decline in sensitivity to fenpropidin relative to that for fenpropimorph (F. J. Burnett—unpublished data). The genetics of cross-resistance to the morpholines in *E. graminis* f.sp. *tritici* are not understood.

4.3 Fitness and stability of isolates with reduced sensitivity

In the absence of fungicide the proportion of isolates with reduced sensitivity falls,[23,24] which would imply that they are less fit. However, they could still be detected, though at low numbers, with the premise that, once insensitive isolates arise, they may persist at low number in the population even where there is no selective advantage.

5 CONCLUSION

In conclusion, sprays of fenpropimorph did cause a significant reduction in the sensitivity of mildew isolates assayed in a field trial, but this reduction was not influenced by the rate of fenpropimorph applied. Reduced doses can therefore be used where applicable without increasing the risk of insensitivity to fenpropimorph. Research has shown, however, that the number of applications should be kept to a minimum and multiple application should probably be avoided wherever possible. In keeping with previous observations, fenpropimorph was found to retain its effectiveness as a fungicide for the control of wheat powdery mildew in the field.

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